EFFECT OF A DIFFERENT RATE OF GROWTH OF ANOXIA ON THE VEGETATIVE FUNCTIONS AND BIOELECTRIC ACTIVITY OF THE BRAIN OF A DEVELOPING ORGANISM

I. G. Dallakyan and G. M. Nikitina

As yet but little study has been devoted to the significance of the rate of increase of oxygen deficiency in the development of compensatory reactions and to survival of the organism in the case of anoxia. There are indications in the literature that at a slower rate of environmental atmospheric rarefaction the stability of the organism is higher than at a rapid rate of development of anoxia [1]. An opposite relationship has been discovered, however, in a number of cases [13]. /256

Investigations with human beings have revealed that at extraordinarily low rates of increase of decompression (tens of hours), the "altitude ceiling" turns out to be lower than at high rates.

Within the framework of clarifying the compensatory mechanisms of the central nervous system in the case of anoxia it is of interest to study the features of bioelectric response of the brain. The fundamental electrophysiological investigations are concerned with the effect of acute oxygen starvation in the case of anoxic anoxia on the EEG of a mature organism [9, 11, 15].

Some data on the character of the change in electrical activity of the brain during various degrees of anoxia in animals and man at various age levels have been given in [3, 4].

Ye. L. Golubeva [3, 4] describes the genesis, in the reticular formation of the midbrain and of the optic thalamus of rabbits, of synchronized rhythm (4 - 7 oscillations/sec) when the CO_2 content in the inspired air is from 1-10%. This synchronized rhythm was more pronounced after the fifth or sixth day of life. In the cortical regions of the brain the synchronized activity observed in rabbits in the first few days after birth changes to unsynchronized activity after the sixth to tenth days. The author arrives at the conclusion that this represents early maturing of the mechanisms which maintain the internal medium of the organism constant.

In all the cited papers dealing with the electrical activity of the brain in the case of oxygen deficiency of the mature and developing organism the method of rapidly increasing anoxia was used.

At the same time, a study of the characteristics of variation in the bioelectric activity of various brain structures during slowly increasing anoxia may be of considerable importance in solving the problem of the compensatory capabilities of the organism during prolonged space flights.

In solving this problem the ontogenetic aspect of the investigation should provide good assistance, since it is easier to clarify the fundamental mechanisms of compensatory reactions of the integral organism to anoxia during the formation of gradually more complex forms of reactions and of relations within the central nervous system. /257

The aim of the present investigation is to study the dynamics of changes in the bioelectric activity of a number of regions of the brain and of the vegetative functions of a developing organism at a varying rate of increase of anoxic anoxia.

Investigational Procedure

The investigation was carried out in chronic experiments on 30 rabbits varying in age (from 4 to 90 days). The anoxia was produced in a pressure chamber at two rates of "climb"—2 and 15 m/sec. During the experiments we measured:

a) the character, sequence and depth of changes in the bioelectric activity of regions of the neo- and archi-pallium (the motor, optic, the anterior and posterior limbic regions, of the neopallium, and the hippocampus) and of a number of subcortical nonspecific formations of the thalamus, of the posterior hypothalamus, the reticular formation of the midbrain, all of which form part of the limbic system of the brain;

b) the relationship between changes in EEG and vegetative functions and respiration and cardiac activity.

Biopotentials were elicited by electrodes incorporated bipolarly at symmetrical points of the hemispheres.

The subcortical Ni-Cr electrodes were machined electrolytically [5] to a point diameter of 100-150 microns and were inserted by means of a stereotaxic Horsley-Clark instrument. The diameter of the cortical electrodes (extradural) was 300 microns. The coordinates of the selected points were determined by the stereotaxic charts of Sawyer, Everett and Green with appropriate recalculations for the species and age of the test animals [10].

In order to make a control test, the brains of the animals coming from the experiment were fixed in a 10% solution of formaline, and then sections 120-150 microns thick were cut on a freezing microtome (Figure 1). The respiration was recorded by means of a thermistor [2], and the EKG by means of silver-foil electrodes.

After the period of adaptation of the animal to the environmental situation has expired (15-20 min) the initial background of respiration, EKG and EEG is recorded, and then "altitude" is reached at the given rate. The functions being studied are recorded at each 1000 m of "climb" until respiration ceased or until there is an abrupt interruption of cardiac activity. "Descent" from the final altitude was at a rate of 100-150 m/sec. In part of the experiments records

were made continuously during the entire descent period until the indices being studied were recovered.

/258

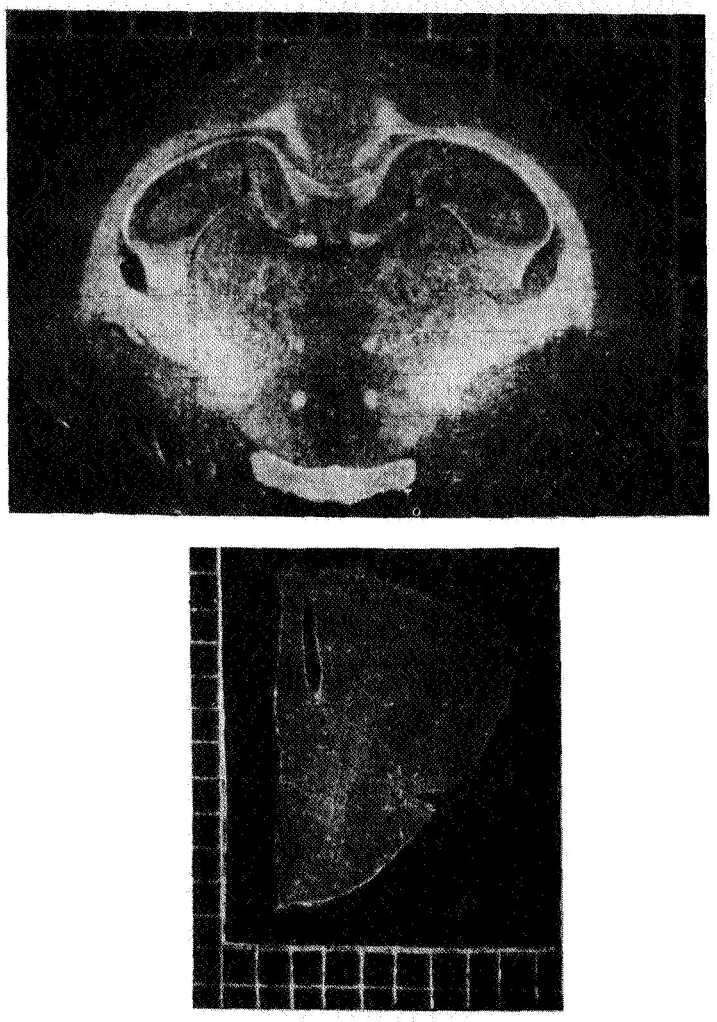

Figure 1. Morphological Control of the Correctness of Arrangement of the Electrodes. Traces of the Tips of the Electrodes in the Hippocampus and Anterior Limbic Cortex are Visible in the Frontal Section of the Brain of a 37-Day-Old Rabbit.

/259

In control experiments on 17 rabbits of different age the pattern of bioelectric reaction of the brain and the vegetative components were monitored for 2-5 hours without the effects of anoxia.

Results of the Investigations

In rabbits up to 20 days old the difference in magnitude of the "altitude ceiling" at the two "climb" rates used is statistically reliable: the rarefaction rates were 2 m/sec for 13,000 m and 15 m/sec at 11,000 m. In rabbits 35-90 days old the altitude ceiling is not reliably (P = 0.05) different in magnitude at "climb" rates of 2 and 15 m/sec (10,610 and 10,900 m), respectively (Table 1). /260

TABLE 1. Comparative Data of the Variation in Altitude Stability of Young and Old Animals at Various Climb Rates

Climb at a rate of 2 m/sec				Climb at a rate of 15 m/sec			
No. of animals	Age (days)	Altitude stability		No. of animals	Age (days)	Altitude stability	
		Time (min)	Ceiling (m)			Time (min)	Ceiling (m)
			Age group (young)				
6	4	98	11,800	6	7	13	11,000
12	6	108	13,000	13	10	10	9500
13	9	97	11,500	29	13	14	11,700
14	12	127	15,250	30	19	14	12,000
14	15	108	13,000				
15	17	116	13,900				
Average		109	13,000	Average		12	11,000
			Age group (old)				
7	30	78	10,000	23	27	13	11,500
9	32	89	10,500	29	31	15	13,000
3	35	76	9500	27	48	11	10,000
4	37	83	10,700	20	58	12.5	11,800
11	41	104	12,500	10	48	13	12,000
3	49	100	12,000	21	60	12	11,000
10	50	94	11,300	27	45	11.5	10,400
21	53	85	10,300	98	78	11	10,000
22	85	83	10,000	21	64	11	95,000
25	48	83	10,000				
26	45	83	10,000				
Average			10,610	Average			10,960

At rarefaction rates of 2 m/sec, the first hypoxic reaction in rabbits of all ages is exhibited by the function of respiration. A change in respiration appeared already at altitudes of 1000-3000 m (Fig. 2A); it is manifested primarily by use in frequency of respiratory motion and a decrease in amplitude of motion. In rabbits up to 20 days old the respiration frequency increases during the period on the average by 10-20%, and from 30 to 70% in the older rabbits. /261

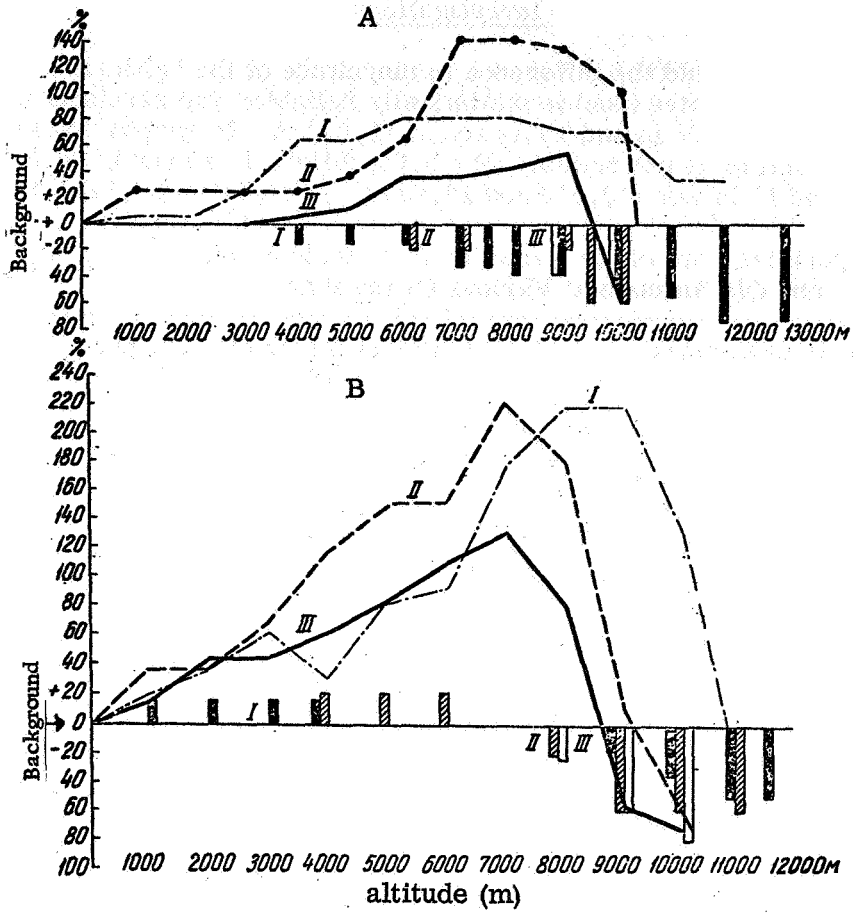

Figure 2. Dynamics of Measuring the Respiration (curves) and Cardiac Activity (columns) in Rabbits Aged (in days) 6 (I), 45 (II), and 85 (III) with Increasing Anoxia to a Rarefaction Rate of 2(A) and 15 m/sec (B).

At altitudes of 4000-9000 m changes in cardiac activity appears in the form of a gradual decrease in heartbeat frequency (Figs. 2A and 3A). In the early age period the disruptions of cardiac rhythm appear earlier (4000-6000 m), as a rule, than in later stages of development (6000-8000 m). As the degree of oxygen deficiency increases, the depth of deviations in the vegetative functions increases. Characteristics of the lower age groups is the parallel increase in the respiration rate and attenuation of the heartbeat; in the higher age groups (after 20 days), however, the heartbeat frequently slows down earlier, in the period when the reaction of the respiration rate drops (Fig. 2A).

In the case of rarefaction at a "climb" rate of 15 m/sec, a similar sequence of inclusion in the hypoxic reaction of the vegetative functions is noticed only in rabbits of the higher age groups (35-90 days) [Fig. 3B]. In the younger animals the variations in EKG and respiration appear simultaneously (from 1000-2000 m), and the intensity of the respiratory reaction is almost double that at a "climb" rate of 2 m/sec (Figs. 2A and B).

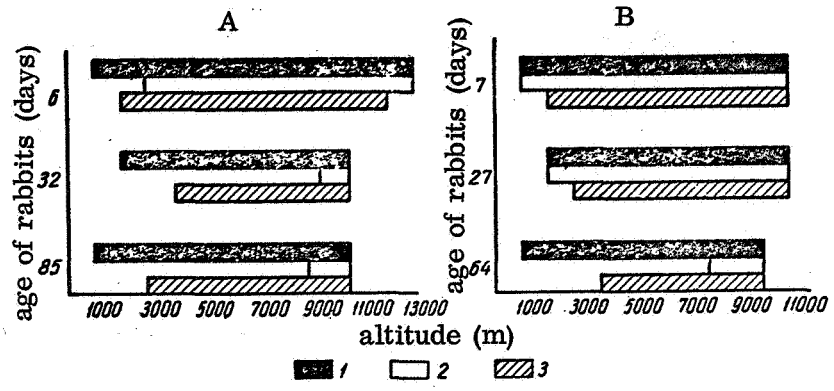

Figure 3. Sequence of inclusion in the hypoxic reaction of respiration (1), cardiac activity (2) and bioelectric cerbral activity (3) in rabbits of different ages at a "climb" rate of 2 (A) and 15 m/sec (B).

Changes in the cardiac activity at "climb" rates of 2 and 15 m/sec are different in character: in the first case the anoxic reaction shows up from the very start as damping of the heartbeat, and in the second case the original phase (up to 7000 m) is an increase in rate of heartbeat (Figs. 2A and B); in older rabbits more intense qualitative changes in EKG are observed (atrioventricular blockade, scintillating arrythmia, palpitation of the ventricles). /262

Initial variations in EEG of the cerebral regions being studied appear in rabbits up to 45 days old with a "climb" rate of 2 and 15 m/sec at exactly the same altitudes (2000-3000 m). Changes in EEG with age at a climb rate of 15 m/sec begin to appear only beginning with 4000-5000 m (Figs. 3A and B).

The dynamics of changes in EEG in young rabbits (up to 20 days) at a climb rate of 2 m/sec consist of 6 successive phases: I - decrease in amplitude and, in a number of cases, damping of the oscillation frequency; II - appearance of groups of synchronized waves of the spindle type (4-7 Hz); III - increasing reduction in amplitude and damping of rhythm; IV - predominance of slow waves (2-4 Hz) of relatively low amplitude, the appearance of spikes; V - alternation of electric activity with periods of electric silence; VI - complete electric silence (Fig. 4).

In older rabbits subjected to advancing anoxia at the given climb rate, the I stage is manifest in a rise of oscillation amplitude connected with damped rhythm (Fig. 5-1, a, b); a constant synchronized rhythm of 7-8 Hz appears in the second stage; characteristic in the IV stage in the appearance of high-amplitude (up to 300 microvolts) slow (0.2 - 1.5 Hz) waves; stages IV, V and VI are of short duration and follow each other rapidly (Fig. 5, 1 c, d, e).

In young rabbits (up to 20 days) at a "climb" rate of 15 m/sec, in contrast with the corresponding group of animals at a "climb" rate of 2 m/sec, the successive EEG phases appear at high altitudes, except for the first phase of initial changes. Thus, phase II of characteristic synchronized waves (spindle-shaped groups) in the case of rapid development of anoxia (15 m/sec) shows up

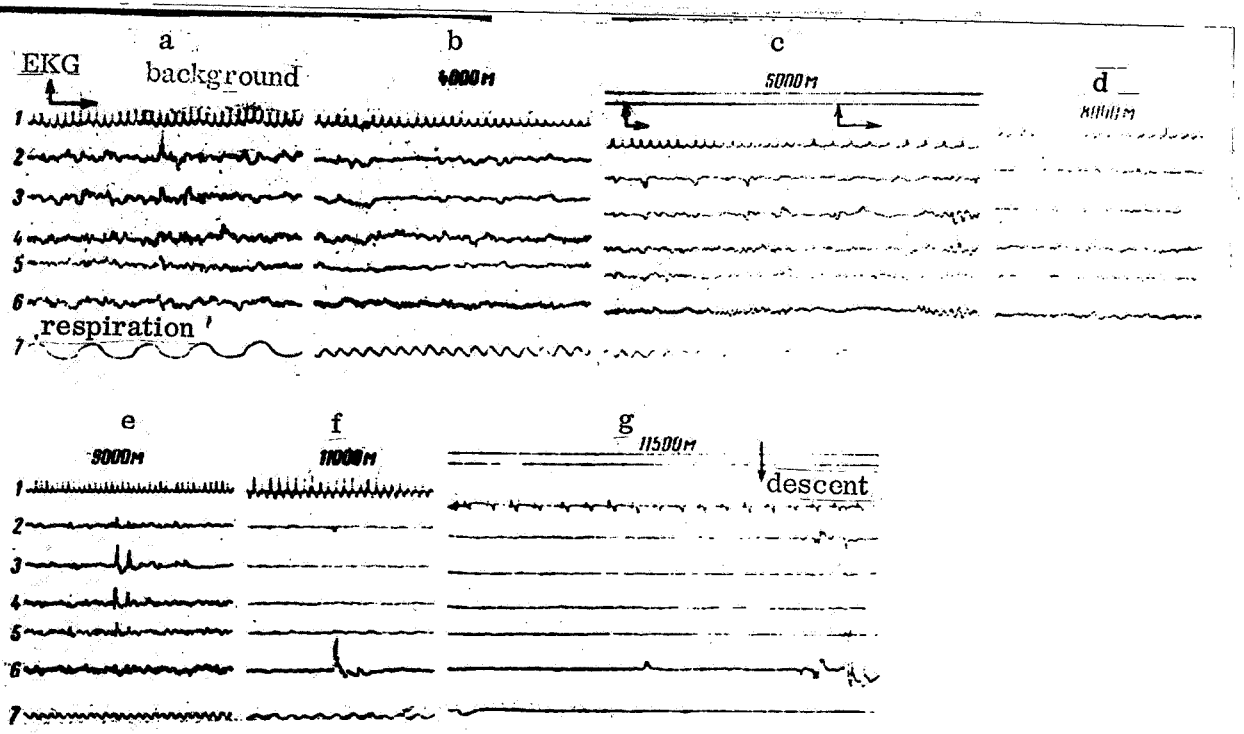

Figure 4. Dynamics of Phase Variations in Bioelectric Activity of the Cerebral Regions Being Stuided for Thirteen Rabbits (the Experiments Began at the age of 4 Days) at a Climb Rate of 2 m/sec. a-Background; b-f-Phases I to VI; g-Phase II at a Rarefaction Rate of 15 m/sec (6 Rabbits, Experiments Began at the age of 7 Days); 1-EKG; 2-6-EEG, Sensomotor, Anterior Limbic Cortex, Hippocampus, Hypothalamus and Reticular Formation of the Midbrain, Resp., 7-Pneumo-gram. Calibration is 50 Microvolts, 1 sec.

in the EEG at an altitude of 8000 m; at the slow rate of rarefaction (2 m/sec), this phase appears already at an altitude of 6000 m (Fig. 4, c, g). The stage of pronounced "spike" waves is absent in the majority of cases.

In rabbits of the older age group, phase changes in EEG characteristic of the 2 m/sec rate are reduced (at the 15 m/sec rate): the initial changes in bioelectric activity are manifested in a reduction of amplitude, increate in rhythmic frequency accompanied by the simultaneous appearance of a synchronized rhythm of 6-8 Hz (Fig. 5, II, a, b); the three subsequent phases (slow waves, alternation of and suppression of electric activity) are very fast. Absolute electric silence does not show up in some cases, even when respiration completely stops. In the last phases the qualitative changes in cardiac activity, namely, atrioventricular blockade, scintillating arrhythmia, palpitation of the ventricles, seldom occur (Fig. 5, II, c, d).

Against a background of increasing phase changes in the EEG in the case of slowly advancing anoxia (2 m/sec) there occur periods in which the electric activity returns in frequency or amplitude to the original value. Such "compensation" is unusually noted at altitudes of 4000, 7000 and 10,000 m, but more frequently at altitudes of 6000-7000 m, which corresponds to the period in which the compensating capabilities of the vegetative functions show up as being more pronounced. Periods of "compensation" of bioelectric cerebral activity also are found at the higher rate of "climb" (15 m/sec) at altitudes of 7000 and 9000 m. /265

The bioelectric reaction of the brain (all the described phase changes of the EEG) at a "climb" rate of 2 m/sec frequently occurs in the cortex regions, the sensomotor region of the neocortex and the anterior limbic cortex, which is morphologically related to the former [14]. With an increase in the degree of oxygen deficiency the changes in EEG appear in the subcortical structures, the reticular formation, the posterior hypothalamus, and also in the region of the hypocampus (archipallium) and posterior limbic subregion of the neocortex. Most stable to the more intense levels of anoxia are the structures of the posterior limbic cortex and of the posterior hypothalamus, where changes evolve in parallel.

In contrast, at a "climb" rate of 15 m/sec it is not possible to find out which structures are most stable when the phases change rapidly in succession. Changes in EEG occur almost simultaneously in the cortical and subcortical structures.

Restoration of the investigated vegetative and EEG indices after the action of anoxia takes place in the following order: first the cardiac activity is restored, then the respiration, and finally the bioelectric activity of the brain.

The pattern of restoration of the bioelectric activity of the brain after the effect of anoxia at a rarefaction rate of 2 and 15 m/sec has a "specular" nature; the background EEG is restored through the slow and synchronized wave phases, which are particularly clearly pronounced in the reticular formation, the hypothalamus and the hippocampus (Figs. 6, Ib and IIa).

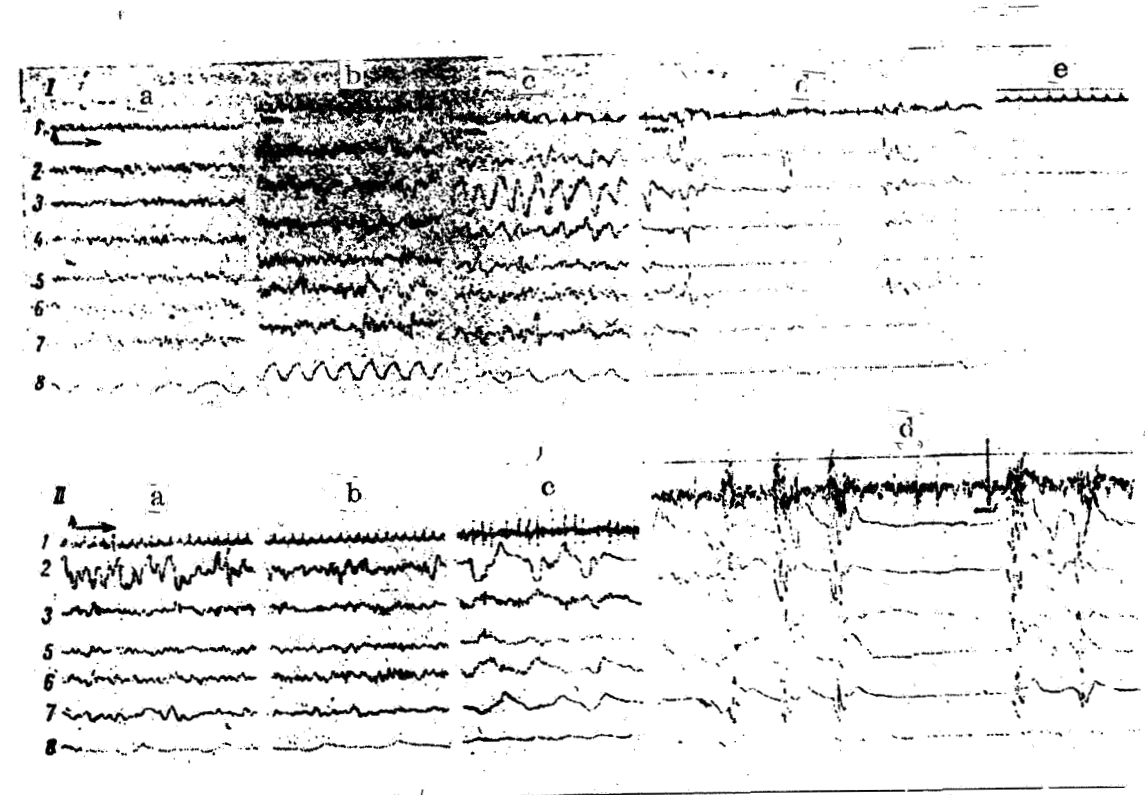

Figure 5. Comparative Characteristics of the Evolution of Phase Changes of EEG in 10 and 23 Rabbits of Higher age (30-50 Days) at a "Climb" Rate of 2(I) and 15(II) m/sec. a-Background; b-I; c-e-EKG; 2-7-EEG of the Sensomotor, Anterior Limbic, Posterior Limbic Regions of the Cortex, Hippocampus, Hypothalamus, and Reticular Formation of the Midbrain, Resp; 8- Pneumorgram; Calibration—50 mV, 1 sec.

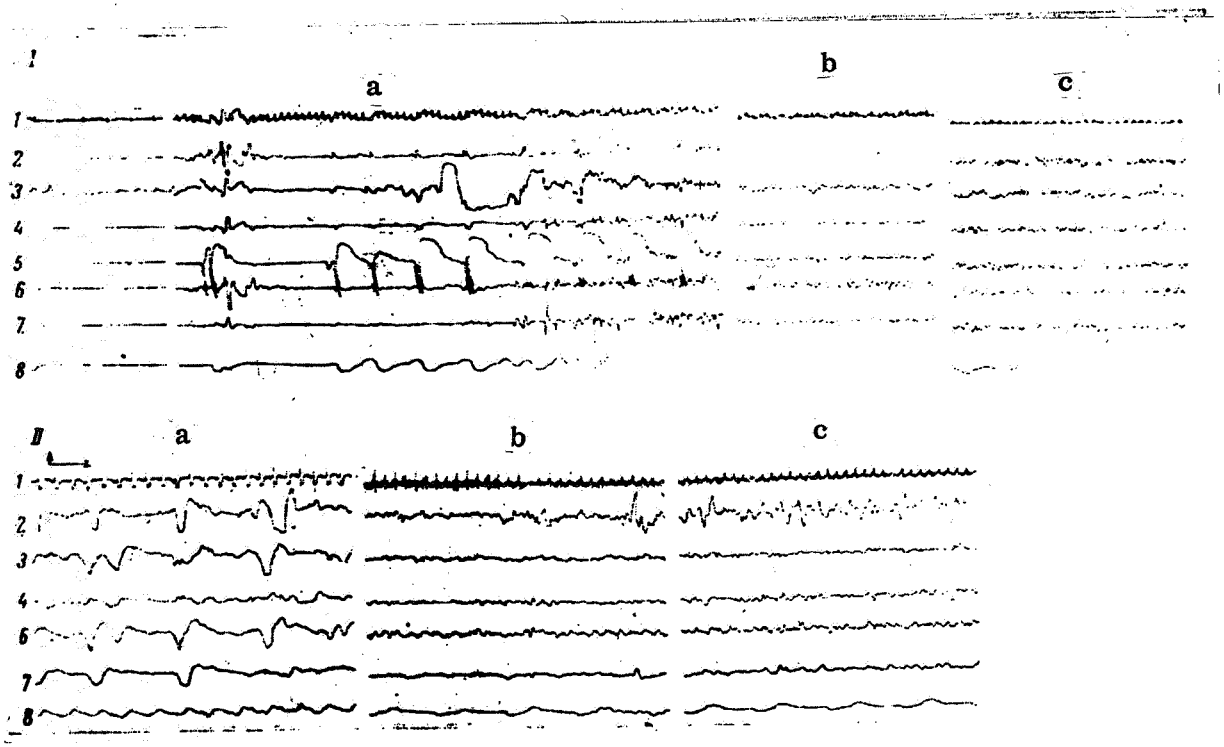

Figure 6. Dynamics of Restoration of Bioelectric Cerebral Activity in Older Rabbits After Anoxia Evolving at a "Climb" Rate of 2(I) and 15(II) m/sec. a-During Descent. The Notation for the Lines in I a-c is the Same as in Fig. 5; in II a-c: 2-Optical Cortex; 4-Thalamus.

In order to restore the background electric activity in young rabbits, as compared with old ones, more time is required both at a "climb" rate of 2 and 15 m/sec; in the latter case, however, the break in time of restoration is less significant.

In animals of the same age, restoration of the bioelectric activity when the rarefaction rate was 15 m/sec proceeded somewhat faster than when the rate of advance of anoxia was 2 m/sec.

Conclusion

These investigations show that the adaptive reaction of the organism to anoxia from the very early stages of positive evolution is complex, consisting of a number of functional components. It covers both vitally important vegetative functions, namely, respiration and cardiac activity, and bioelectric processes in the cerebral tissue. Variations of the vegetative indices and biopotentials in various cerebral structures of developing animals are characterized by phase-type evolution namely, successive transition from one phase of the anoxic reaction to the next, all the way to complete disruption of the functions being studied when the oxygen deficiency reaches low levels. The relationship between the various functional indices (vegetative changes and variations of EEG) is examined for a mature organism [9]. Insofar as the features of the indicated correlations in the light of age are concerned, papers are available from Czech authors (Ilec, Maurec, Fischer, and Krulik) in which the emphasis is on the study of the biochemical structural and functional changes. /267

Both the age characteristics of evolution and the factor of rate of increase of anoxia have on effect on phase changes of the functions we studied.

The singular nature of the original phase of variations in EEG during the early days of growth is manifest not in the activation of high-frequency oscillations, as in the case of a mature organism [9], but in the attenuation of the rhythm. In older animals, as compared with younger ones, the final phases of suppression of bioelectric activity and the accompanying serious disruption of the vegetative functions are seen to be of lesser duration. Better differences in EEG, namely, reactions to anoxia in the cortical and subcortical structures, are observed with age. All this is governed by the degree of maturity of the nervous regulatory mechanisms in this or that stage of autogenesis.

The rarefaction-rate factor makes a considerable contribution to the development of compensatory reactions of the growing organism: the faster the rarefaction, the later (at higher altitudes) intense changes in the bioelectric activity of the brain and in vegetative functions appear. This is especially true of older animals, which have a sufficiently mature central nervous system and more perfect compensatory mechanisms. Therefore they have almost no difference in magnitude of the altitude ceiling at the two given rarefaction rates (2 and 15 m/sec). In contrast, young animals have a considerably lower altitude ceiling at the faster rate of increase of anoxia because their adaptive mechanisms are incomplete.

At the rarefaction rate of 15 m/sec the compensatory reaction, estimated on the basis of EEG, usually involves a large number of cerebral structures simultaneously. In contrast, at the slower rate of increase in anoxia (2 m/sec) the time sequence of onset of changes in the cortical and subcortical structures is more clearly pronounced. The above-mentioned fact of synchronization of changes in the bioelectric activity of the hypothalamus, hippocampus, and posterior region of the limbic cortex indicate that structures of the limbic system of the brain participate in the defense reaction of the organism as a unified functional system. The unified participation of the nervous mechanisms of this system in maintaining functions important for the vital activity of the organism at a specific level in the case of oxygen deficiency is ensured by the existence of morphological and functional links between the indicated cerebral structures [6-8, 11].

/268

REFERENCES

1. Vladimirov, G.Ye. Fiziol. Zh. SSSR; 25, 779 (1938).
2. Volokhov, A.A., Kobysh, V.I. and Novikova, Ye.G. Zh. vysshey nervnoy deyat.; No. 2 (1956).
3. Golubeva, Ye.L. Tez. i ref. dokl. 3-go nauchn. soveshch. po evol. fiziol. [Reports and Reviews of Reports at the Third Scient. Sym. on the Evolution of Physiol.] Leningrad (1961).
4. Golubeva, Ye.L. Fiziol. zh. SSSR; 48, 10 (1962).
5. Gusel'nikov, V.M. Zh. vysshei nervnoi deyat.; 7, 626 (1957).
6. Dykman, L.M. Materialy IV elektrofiz. konf. [Reports of the IVth Electro-Phys. Conf.]; Rostov (1963), p. 139.
7. Zambrzhitskiy, I.A. In the book Razvitiye tsentral'noy nervnoy sistemy [Development of the Central Nervous System]; Moscow (1959).
8. Zambrzhitskiy, I.A. Arkhiv anat., gistol. i embriol.; 1, 23 (1966).
9. Malkin, B.V. Razumeyev, A.N. and Izosimov, G.V. In the book Kislorodnaya nedostatochnost' [Oxygen Deficiency]; Kiev (1963), p. 104.
10. Meshcherskiy, R.M. Stereotoksicheskiy metod [Stereotoxic Method]; Moscow (1961).
11. Nauta, B. In the book Mekhanizmy tselogo mozga [Mechanisms of the Entire Brain]; Translation, Izd. IL (1963), p. 182.
12. Parfenova, O.I. and Livanov, M. N. Byull. eksperim. biol. i med.; 20, No. 3, 36 (1945).
13. Petrov, I.R. Kislorodnoye golodaniye golovnogo mosga (eksperimental'nye materialy) [Oxygen Deficiency in the Brain (Experimental Data)]; Leningrad (1940).
14. Sotnichenko, T.S. Arkhiv. anat., gistol. i embriol.; 43, No. 8, 3 (1962).
15. Shpil'berg, P.I. Byull. eksperim. biol. i med.; 18, No. 9, 55 (1943).